

*Clostridium difficile*

## B

**Detection of Toxin B Gene of *Clostridium difficile* by Polymerase Chain Reaction from Clinical Isolates**

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**Background :** *Clostridium difficile* causes antibiotic-associated diarrhea or pseudomembranous colitis by producing of toxins in patients treated with antimicrobial agents. Stool cultures for *C. difficile* and tests for the presence of its toxin are the most widely used methods for the diagnosis of infection. The aim of this study was to determine the usefulness of polymerase chain reaction for the detection of toxin B gene from *C. difficile* isolates.

**Methods :** In this study, 85 strains of *C. difficile* were used, which were isolated from stool specimens of patients with suspected antibiotic-associated diarrhea or pseudomembranous colitis from 1987 to 1994 using cefoxitin-cycloserine-fructose agar. DNA of the *C. difficile* isolates was extracted by boiling and by conventional methods. The primers used for toxin B gene amplification were YT-17, 5'-GGTGGAGCTTCAATTGGAGAG-3' and YT-18, 5'-GTGTAACCTACTTTCATAACACCAG-3'. Amplification products were electrophoresed in a 1% agarose gel containing ethidium bromide and the presence of the 399 bp band was examined under ultraviolet light. The results were compared with those of toxin A detection by PCR and with the results of quantitative cultures.

**Results :** Toxin B gene was detected in 74% (63/85) of the *C. difficile* isolates. Toxin B gene was detected in all strains with toxin A gene, but not in the strains without toxin A gene. DNA extraction by boiling and by conventional methods gave the same detection rate. The positive rate of toxin B gene was slightly higher in the strains which were isolated with a higher colony count from stool than nontoxigenic ones.

**Conclusions:** The PCR detection of toxin B gene is a useful method for identifying the toxigenic *C. difficile* strain in the clinical laboratory, and the boiling method is simple for DNA extraction. The use of a toxin test can reduce false positive diagnosis due to the presence of nontoxigenic strains among the isolates.

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**Key words :** *Clostridium difficile*, Toxin A, Toxin B, Enterotoxin, Cytotoxin, Polymerase chain reaction

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*Clostridium difficile*

A (enterotoxin) B (cytotoxin)

[1]. clindamycin, ampicillin, cephalosporin

, 가

가 [2, 3]. C.

*difficile* 1989 6% (8/142)

1997 29% (176/609) 가

[4].

[3, 57],

10-20%가

[3, 5-7].

*C. difficile*

. 가 ,

가

[9].

*C. difficile*

[10] (polymerase chain reaction, PCR)

[11-13]. 가 ,

가

*C. difficile*

PCR B

. *C. difficile*

1987 1994

. *C. difficile*

0.1 mL

thioglycollate 10 3

, 0.1 mL

cycloserine-cefoxitin-fructose agar (CCFA)

24-48

35

ATB 32A Kit (bioMerieux SA, Marcy l'Etoile, France)

. 1 mL

. 85 C.

*difficile* *C. difficile*

*Clostridium* spp., *C. clostridioforme*, *C. perfringens*, *C. septicum*, *C. tertium* 2 *C. butyricum*, *C. histolyticum*, *C. innocuum*, *C. sordellii*, *C. sporogenes*, *C. tetani* 1 ,

*Escherichia coli*, *Klebsiella pneumoniae*

*Pseudomonas aeruginosa* 1 .

A PCR Kato [12]

Gifu Anaerobe Institute . B

PCR Gumerlock

[13] . *C. difficile* DNA

, 21 [14] , 64

가 [15, 16] .

*C. difficile* 50 mL brain

heart infusion (BHI) broth 37 48

6,000 x g 20 50 mM

Tris (pH 7.4) . 2 mL Tris-borate-

EDTA (TBE) buffer (pH 8) . Lysozyme

0.5 mg/mL가 가 , 37 15

sodium dodecyl sulfate (SDS)

0.5%가 가 60 10 .

1,000 x g 10

phenol-chloroform-isoamyl alcohol (24:25:1)

95% cold ethanol 가

-20 -70 1 DNA

Tris-EDTA buffer (pH 7.4)

PCR . 가 2-3

100 µL 10

, 13,000 x g 1 30

PCR .

B YT-17:

5'-GGTGGAGCTTCAATTGGAGAG-3' YT-18:

5'-GTGTAACCTACTTTCATAACACCAG-3'

[12]. PreMix- Top (Bioneer, )

DNA 1 µL 1 µL (20 pmole)

17 µL .



Fig. 1. Detection of toxin B gene of *Clostridium difficile* by PCR. Lane 1, negative control (D.W.); lanes 2 and 9, size marker (123 bp); lane 3, positive control strain; lanes 4 to 8, toxin B-positive strains; lane 10, toxin B-negative strain; lanes 11 to 17, toxin B-positive strains.

Table 1. Comparison of two DNA extraction methods for toxin B gene detection by PCR

Strains with toxin A	Toxin B detection with extraction by:					
	Conventional method*(%)			Boiling method† (%)		
	Positive	Negative	Total	Positive	Negative	Total
Positive	12 (57)	0 (0)	12 (57)	51 (80)	0 (0)	51 (80)
Negative	0 (0)	9 (43)	9 (43)	0 (0)	13 (20)	13 (20)
Total	12 (57)	9 (43)	21 (100)	51 (80)	13 (20)	64 (100)

\*DNA extraction by Gumerlock et al.[15, 16]. Concordance rate of toxin A and B gene detection by PCR was 100%.

†DNA extraction by Tang et al.[14]. Concordance rate of toxin A and B gene detection by PCR was 100%.

Table 2. Comparison of quantitative culture results with toxin B gene detection rate by PCR

<i>C. difficile</i> in stool (CFU/mL)	No. of strain tested	Toxin B*	
		Positive	Negative
10 <sup>6</sup>	39	33 (85%)	6 (15%)
10 <sup>2</sup> – <10 <sup>6</sup>	20	12 (60%)	8 (40%)
Total	59	45	14

\*Significance of difference ( $P = 0.037$ , Mann-Whitney's one-sided method).

95 5 , 95 30 , 58 30 , *C. difficile*  
 72 30 50 . 10 µL *C. difficile* . *C.*  
 0.5 µg/mL ethidium bromide가 1% agarose *difficile* CCFA  
 gel , 399 bp 가 16S rRNA PCR  
 (Fig. 1). [19].  
 . 16S rRNA *C.*  
*difficile* PCR  
 가 85 63 4-5 PCR  
 (74%) . A 가 [19]. 가 , PCR  
 B 가 , A 가 , PCR  
 22 (26%) B *C. difficile* [9].  
*Clostridium* ,  
 B 가 . A 가 .  
 DNA 가 .  
 (Table 1). 1 가  
 59 1 g [9].  
*C. difficile* PCR 4-6  
*C. difficile* 가 A B  
 (p=0.037, Mann-Whitney's one-sided method, Table 2). 가 [13]. A 가  
 PCR A 가  
*C. sordellii* 가 [20, 21]. B  
 PCR ,  
*C. difficile* *Clostridium* spp.  
*E. coli*, *K. pneumoniae*  
 [17]. 가 . 85  
 , A 가 B *C. difficile* B  
 가 [18]. , 74% (63/85)가 , 2/3

- [3]. *C. difficile* 30% : *Clostridium difficile* A B
- , *C. difficile* 가 [3], B
- 가 [22-24] *difficile* (PCR) B
- [18]. , 가 : 1987-1994
- 31.8% [23] *C. difficile* 85
- A B 가 DNA ,
- 가 가 PCR YT-17,
- 5'-GGTGGAGCTTCAATTGGAGAG-3' YT-18,
- 5'-GTGTAACCTACTTTCATAACACCAG-3'
- 95 30 , 58 30 , 72 30 50
- , 1% agarose gel 399 bp
- PCR A
- [14],
- 가 ,
- : 85 63 (74%) B 가
- . PCR A 가
- B 가 , A
- 가 22 (26%) B
- DNA , 1 g *C.*
- difficile* 가
- : *C. difficile* B
- PCR
- DNA 가
- PCR 2-4
- PCR
- heme, bilirubin, urobilinogen, bile salt DNA
- [26] immuno-
- magnetic enrichment[27], phenol-chloroform technique[28]
- PCR
- C. difficile* B
- PCR
- DNA 가
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*Clostridium difficile*  
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